COMPARATIVE VIEW OF THE PRINCIPAL BACTERIA OF DISEASE. (AFTER CORNIL AND BABES.)

XI035.

REFERENCES TO PLATE XI035.

1. Anthrax, a. b. c d and a. b. c. d. e. f g. h.
2. Chicken Cholera, a. b c.
3. Glanders, a b c,
4. Typhoid Fever, a. b.
5. Erysipelas,
6. Puerperal Fever.
7. Yellow Fever, a. b.
8. Measles, a b. c
9. Diphtheria, a. b. c. d.
10. Pneumonia.
11. Hog Cholera, a. b.
12. Variola.
13. Vaccinia.
14. Noma.
15. Gangrene, a. b.
16. Gonorrhœa, a b.
17. Septicæmia of Mice, with Kidney Lesions.
18. Pyæmia.
19. Endo-carditis.
20. Parenchymatous Nephritis.
21. Cerebro spinal Meningitis.
22. Tuberculosis, a b. c.
23. Tuberculosis, d. e.
24. Leprosy, a. b. c. d.
25. Whitlow.
26. Red Sweat.
27. Wart.
28. Jequirity, a. b. c. d. e. f. g. h. i.
29. Recurrent Fever.

AN INTRODUCTION

TO

PRACTICAL BACTERIOLOGY.

A GUIDE FOR

STUDENTS AND GENERAL PRACTITIONERS.

BY

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1887.
GEORGE S. DAVIS,
DETROIT, MICH.

TABLE OF CONTENTS.

BOOKS OF REFERENCE:

Les Bacteries. Cornil & Babes. Paris, 1885.

Micro-Organisms and Diseases. Klein. London, 1885.

Pathological Mycology. Woodhead & Hare. Edinburgh, 1885.

Die Micro-organismen. Fluëgge. Leipsig, 1886.

Practical Bacteriology. Crookshank. New York, 1886.

Bacteriologische Untersuchungs-Methode. Huber & Becker. Leipsig, 1886.

PREFACE.

It has been the aim of the writer in issuing this little monograph to furnish the student and medical practitione with a concise resumé of bacteriology, practical in character, and so extend more widely an interest in this most important topic.

A single chapter has been devoted to the subject of Germ Theories, and to the successive advances that have been made towards securing our present knowledge.

Bacteriology, as a branch of medicine, has already obtained for itself a name and permanent place, in spite of the many obstacles and the vigorous opposition it has encountered; and yet it will be conceded by the most ardent bacteriologist that many of its fundamental principles are shrouded in obscurity.

But there is good reason to believe that the researches of the next few years will yield rich results, for steady progress is now being made towards the perfection of those instruments of precision that are necessary for future work, and we may confidently hope that the morphology of these microphytes, their proper classification, chemical qualities and physiological attributes will soon be satisfactorily established.

As the scope of this little guide precluded much originality, the writer has made no special efforts in that direction, but has freely borrowed from the subjoined list of works, which may be consulted for many details that were inadmissible for manifest reasons. Thanks are here given to Dr. J. M. Rice for his assistance in preparing the chapters on methods, general and special; also to Messrs Eimer & Amend, of this city, for the use of many electro-plates.

T. E. S.

17 E 44th Street, New York City, April 20, 1887.

CHAPTER I.

INTRODUCTORY.

As prefatory to this subject, I think it advisable to reproduce some of the points of a paper read in Philadelphia, before the International Medical Congress, of 1876, partly because certain conclusions were there maintained in the section on sanitary science, before which the topic was discussed, and because they were further sustained by the Congress sitting as a whole; and also for the reason that they will serve as a fitting introduction to the general subject. And it may be remembered, incidentally, that at that remarkable gathering there were present some of the most prominent advocates* of what was then called the germ-theory. In enumerating at that time the various theories and hypotheses that had been framed to explain the origin of infective diseases, three were given, viz.: (1) the vegetable germ; (2) the bioplasm; (3) the physico-chemical, and they were briefly sketched as follows:

I. It was said that the vegetable-germ theory had attracted most attention, but was a flimsy hypothesis until the microscope came into use. Then it was that Schwann, Cagniard de Latour and Kützing†

* Lister, Hueter.

† Schutzenberger, on Fermentations, pp. 36–7.

observed that there was a growth of microscopic organisms in fermenting fluids, and when at a later date Pasteur maintained that these organisms actually produced the fermentative process, then medical men inquired whether it might not be true that infective diseases had an analogous origin. But the best *a priori* evidence of the truth such theories presented was adduced by Schönlein, who, in 1838, published his account of the microscopic organism found in favus, or honeycomb ring-worm. And he substantiated his views in so able a manner that the plant has come to be held by dermatologists and others as the cause of the disease. It is classified under the moulds or fungi. At the time when the paper before alluded to was read, very many of the infective diseases had been alleged to have a specific microphyte, causing the disease by their own inherent poisonous qualities. Such, for example, was the measles-fungus of Salisbury, the diphtheria micrococcus of Letzerich, the cholera-fungus of Hallier, described in 1867, the microphæræ vacciniæ of Cohn, the bacteridiæ of anthrax, discovered by Pollender and Davaine, the spirillus of relapsing fever, discovered by Obermeier, and the microcytes of typhoid fever, described by Klein, with some others whose claims to recognition were less definite.

II. The second hypothesis was that which prob-

† Bastian, Lancet, April 10, 1875, p. 502.

ably originated with Beale.* He was willing to admit that particles of microscopic size, molecules in fact, would produce disease, but he held that they were degraded portions of the animal system. And yet they had the power of dividing and subdividing under diseased conditions, "as living matters alone divide," to quote his language. The distinguished Hutchinson, of London, held the same view, believing, in particular, that gonorrhœa and purulent ophthalmia, erysipelas and phagedæna were communicated by such living material.

II. The third hypothesis was well expressed at that time by Bastian. It was called the "physico-chemical," or "physical" theory. As then enunciated, it held that though minute organisms might act as ferments, they did so by virtue of chemical actions set up by them, while minute particles of the human body had an almost equal capacity for setting up diseased action under suitable conditions. In reference to the relation between organisms and fermentation, I must be permitted to quote from my own paper, which states:

"After the publication of Pasteur's brilliant experiments in relation to fermentation and putrefaction, they were regarded as affording good *à priori* evidence of the truth of the doctrine now under consideration. But it must be remembered that, though sustained by

* Beale, Disease Germs, pp. 5 and 11.

the observations of many others, these views were strenuously opposed by Willis, Stahl, Liebig, and others, chiefly of the German school.* There is little doubt now that these latter were in a measure correct; in fact, Pasteur† has seen fit to modify some of his earlier statements, for he quite recently has said that both alcoholic fermentation and putrefaction may be initiated by the chemical processes taking place in the tissue-elements of certain fruits and vegetables, independently of the minute organisms supposed to be necessary to the process. Similar statements had previously been made by MM. Le Chartier and Bellamy, who found that, in modified forms of fermentation, independent organisms were generally absent at first, though they often made their appearance afterwards."

In a subsequent public discussion at the same congress, in another section, I maintained this same ground,‡ and showed that there had been and were those who believe that fermentation is not always dependent on the growth and multiplication of living organisims.§

In a recent and most valuable article in which this topic comes up for consideration,‖ Prof. Knapp,

* Schutzenberger, p. 40.

† Lancet, April 10, 1875, p. 508, and Tribune Med., April 1, 1875, p. 321.

‡ Trans. of the Internat. Med. Congress, 1876, p. 544.

§ Handw. d. r. u. a. Chim. M., 1848, p. 232, Proceedings of the Royal Soc., No. 172, 1876.

‖ The N. Y. Med. Rec., Dec. 25, 1886, p. 701.

of this city, cites very excellent evidence to substantiate the view that fermentation is caused by vegetable organisms, and it is proper to state here that most chemists do rank themselves on his side, which is, in fact, the prevailing and popular one. Prof. Knapp alludes especially to the experiments of Schwann, Helmholz, Schroeder, and von Dusch. And he says, "the microscope has shown that all fermenting substances contain yeast plants, and experiments in various ways have demonstrated that no fermentation takes place so long as the fermentative substance is kept free from yeast cells. The presence of microbes thus being an essential factor in fermentation; the definition of fermentation ought to express it; we may, therefore, define fermentation as the decomposition of carbo-hydrates into simple compounds by the agency of living microbes."

But even admitting for the moment that some fermentations are so produced, these statements are far too sweeping; that is, if I understand rightly the phraseology used by some of our best and latest writers; thus Sedgwick* says, "there is another group of obscure chemical phenomena known as fermentations, produced by certain lifeless or inorganized substances." Such are the mineral acids, sulphuric or hydrochloric, which may, under certain circumstances, decompose starch, cane sugar, and some other organic substances into such compounds, without themselves

* Reference Handbook of the Med. Sci., 1886, III, p. 63.

undergoing any appreciable changes. Thus cane sugar is inverted, that is, turned into dextrine and glucose. Pepsine is another example; so is the diastase of malt. And it is a matter of interest that the conversion of cane sugar into grape sugar is done not by the yeast directly, but indirectly by a soluble ferment, which may be extracted from the yeast and will act in its absence. Similar statements may be found elsewhere, and in particular, Prof. Dittmar, of Glascow, describes, under the fermentative agents proved to be of purely chemical nature, not only certain acids, diastase, emulsine, peptine and pancreatine, but erythryzone, a peculiar ferment discovered by E. Schunck, in 1854, and extracted from madder root, and which he found had the power of inducing vinous fermentation in solutions of sugar. All of these ferments, the acids excepted, lose their efficacy at a temperature of about 100° C., in the presence of water.†

I stated in 1876 that Dougall‡ had produced putrefaction without bacteria, and that Hiller§ made statements to the same effect, for, having injected fresh eggs with a fluid containing bacteria, but not putrid, the eggs remained unaffected, which showed that bacteria might be present without decomposition ensuing. These views received further support from Donné and Beauchamp.‖

† Article on Fermentation, in Encyclo. Brit., No. 7, 1879.

‡ Dougall, Brit. Med. Jour., Apr. 24, 1875, p. 557.

§ Centralbl. f. d. med. Wiss., Dec., 1874.

‖ Schutzenberger, p. 225.

Now we are told* that the action of living bacteria is as essential for putrefaction as for fermentation, and that it can be prevented by adopting proper methods, such as the exclusion of air, washing it, filtering it, or by gravitation according to the methods of Pasteur and Tyndall. Nor is this all, for we are told† that putrefaction and suppuration are identical, since it is now alleged that the bacteria of putrefaction will also produce suppuration. But if such be the case, is suppuration always due to a parasite? I will quote Dr. Knapp's statements on the question: Does traumatism of any kind produce suppuration? "I made a series of experiments in Berlin last winter. I performed all the operations that are practiced on the eye, on the one side of a rabbit, with sterilized instruments, in an aseptic way; on the other side the wound was contaminated with an emulsion of a pure culture of some pyogenic fungus. All the former healed by first intention; the latter suppurated with the regularity of a chemical experiment. The coarsest operation," he continues, "the rudest treatment of a wound, will not be followed by suppuration, if only the pyogenic germs are excluded."

As to the question whether chemical agents produce suppuration without the intervention of microbes? Until recently we are told that opinions were divided on this subject, but that latterly the experiments of

* Knapp loc. cit.

† Knapp, loc. cit.

Straus, Scheuerlein, Klemperer and Ruys have demonstrated to almost a certainty, that *bacteria* are the causes of any form of suppuration, and yet Prof. Knapp tells us that many of the most competent bacteriologists are unwilling to admit this statement, though he firmly believes that sufficient evidence has been adduced to show that suppuration in any case depends on the action of microbes.

I will now introduce the conclusions adopted after the reading of my paper, in 1876, at the Congress in Philadelphia:

I. That, as far as inquiry has been made as to the nature of the active principles in infective diseases, it is probable that in a certain number the matter is particulate, or molecular in form.

II. That in regard to the causes of septicæmia, pyæmia, puerperal fever, erysipelas, and hospital gangrene, and those of cholera, vaccine-disease, the carbuncular diseases of men and animals, typhoid and relapsing fevers, and diphtheria, there is not satisfactory proof that they are necessarily connected with minute vegetable organisms.

III. That the real nature of these causes is still uncertain.

It will now be advantageous, I think, at this point, to give a chronological summary of the events that have succeeded one another in the history of bacteriology, from 1876 to date. About the time that the foregoing paper was written, Pasteur had busied

himself with the *serial cultivation* of bacteria, or microbes as he now called them. His object was to isolate each variety completely, and after a series of cultivations in different media, prove the actuality of his views by inoculation upon animals. Robert Koch, of Berlin, followed closely in the wake of Pasteur, and devoted himself to anthrax; while Pasteur took up an entirely new topic, viz., chicken cholera. But his object was not merely to find the real cause of the disease; he also hoped to secure what he termed a *vaccine*, borrowing a name from the modified virus of small-pox, or cow-pox. About the same time Koch* also devoted himself to experiments in septicæmia and pyæmia, by inoculating animals with putrefying vegetable matter.

In 1879 Neisser† found in the discharges of gonorrhœa a peculiar microphyte that he named the gonococcus. In 1880 Eberth‡ proclaimed anew the bacterial nature of the typhoid fever poison. In 1882 Koch§ described his tubercle bacillus and offered the most satisfactory proof of the bacterial origin of pulmonary

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* Koch, Ueber d. Aetiol. d. Wund-infections-krankheiten, Leipsig, 1878.

† Neisser, Ueber den Pilz der Gonorrhœa, Med. Centralbl., 1879, No. 28.

‡ Eberth, Die organismen, etc., beim Typhus Abdom., Virchow's Archiv., Bd. 81, 1880, p. 58.

§ Koch, Die Aetiol. der Tuberculose, Ber. Klin. Woch., 231, 1882.

tubercle that had been made. In 1883 Fehleisen* found a bacillus in erysipelas, which he regarded as pathogenetic, and in the same year Babes† described a special bacillus in lepra.

The year 1883 was remarkably prolific of bacteriological research, for it was then that Friedlaender‡ called attention to the coccus of lobar pneumonia, and Pasteur and Thuillier following Klein substantiated his views as to the bacterial character of the virus in hog-cholera. In 1884 Laveran§ published his studies on the microzymes of malarial fever, and in 1885 Lustgarten‖ gave a special method, by which he claimed that the syphilitic poison could be shown to have a special parasite.

It is generally admitted by bacteriologists that much of the work just alluded to will require revision, a statement that naturally is applicable to almost all new work on any special topic, but it is plain that the evidence is not so strong in any case, as to the bacterial origin of the disease in question, as in the case of anthrax, tubercle alone accepted. Meanwhile it is proper to note that this bacteriological work has, in a measure, been parallelled by physico-chemical researches which have grown out of these studies, or

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* Fehleisen, Die Etiologie d. Erysipelas, Berlin, 1883.

† Babes, Arch. de Phys., 1883.

‡ Friedlaender, Fortschritte d. Med., Bd. 1, 1883.

§ Laveran, Traite d. fievres palusters, 1884.

‖ Lustgarten, Syphilis-bacillus, 1885, Wien.

have been made independently, the tendency of which is to turn our attention back towards the physico-chemical theory, of which a passing notice has already been made in my introductory statements. Some years ago, Gautier, of France, and Selmi, of Bologne, appear to have made a simultaneous discovery of certain chemical substances that are capable of infecting the system by a peculiar poison. Selmi found these substances in the scrapings of skeletons. These matters possessed definite crystallizable and physiological properties. Some of these could be extracted by ether, and some by chloroform. They were organic substances, producing with sulphuric acid a violet-red color, and giving out an odor like hawthorn, when heated. More recently some excellent studies have been carried out on the subject of hog-cholera, in the Bureau of Animal Industry, in Washington, by Drs. Salmon and Theobold Smith,* and their conclusions are that the poison of the disease in question is not merely found in the special microphyte of the disease, but in a peculiar substance that is elaborated by them. The conclusions are as follows:

1. Immunity is the result of the exposure of the bioplasm of the animal body to the chemical products of the growth of the specific microbes, which constitute the virus of contagious fevers.

2. These particular chemical products are pro-

* Proceedings of the Biolog. Soc. of Wash., Vol. III, 1884–6.

duced by the growth of the microbes in suitable culture liquids in the laboratory, as well as in the liquids and tissues of the body.

3. Immunity may be produced by introducing into the animal body such chemical products as have been produced in the laboratory.

At this point I think it well to state that I fully agree with Cornil & Babes, who have written the most elaborate and perhaps the most satisfactory work on the subject, when they recommend the reader to be cautious in his acceptance of much that has been written on bacteriology, and it is for the following reasons: The morphology of bacteria has not been completed, in a botanical sense; the territory in which they live and move is an uncertain one; animal experimentation is a delicate matter, and offers large opportunities for controversy; the technique of this kind of work is peculiarily difficult, even for one who is versed in ordinary laboratory methods, and finally, the problems relating to the chemical nature and properties of the substances elaborated by bacteria have still to be solved.

CHAPTER II.

THE MICROSCOPE AND MICROSCOPICAL APPLIANCES FOR USE IN A BACTERIOLOGICAL LABORATORY.

The Microscope.—It is necessary to have an unusually good instrument for the study of bacteria, and, indeed, systematic work of this kind requires the use of immersion lenses of good quality, together with an achromatic condenser of approved pattern. At the present time, Zeiss, of Germany, stands pre-eminent as a manufacturer of these lenses, and there is reason to believe that he will carry their construction to a still higher degree of perfection. Meanwhile, other German opticians, and some in England and America* make satisfactory glasses. Those that meet with most favor are the $\frac{1}{12}$, $\frac{1}{18}$, and $\frac{1}{25}$, but it should always be remembered that novices in microscopical studies cannot work advantageously with such high powers, and that considerable practice with lower powers and the general technique of the microscope should precede all attempts to achieve success in the higher field of practical bacteriology.

The best model of an achromatic condenser has been furnished by Abbé, but most microscope makers make condensers that are sufficiently good for most work of the kind.

* J. W. Grunow, Optician, of 70 W. 39th St., New York City, makes a good immersion, $\frac{1}{12}$, for $60.

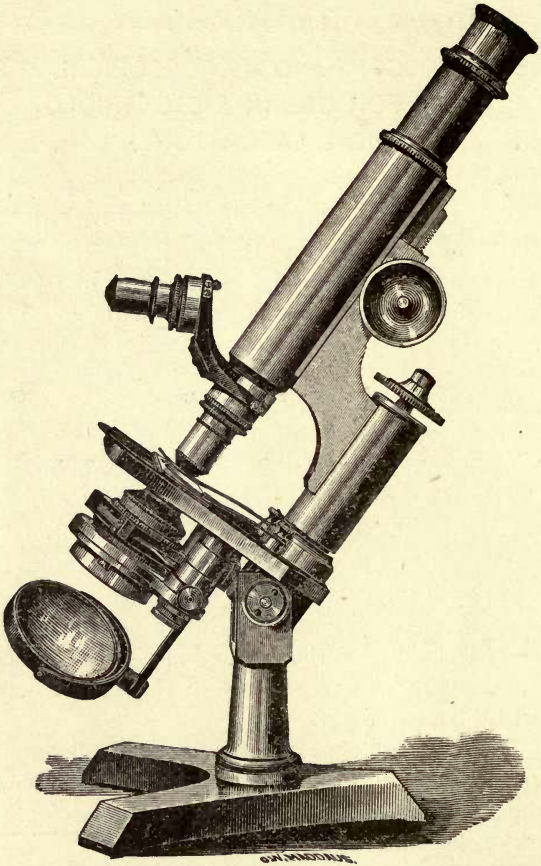

Fig. 1.—A Grunow Microscope for Bacteriological Study, Fitted with Immersion Lenses, Nose-piece, and an Abbé Condenser.

Quite recently Zeiss has introduced novelties in his *apochromatic objectives* and *compensating eye-pieces* for this especial work. In the construction of his lenses a new kind of glass has been made under the supervision of Prof. Abbé. Each apochromatic objective is to be used with a compensating eye-piece, which both corrects the former and gives it greater working distance. The chromatic aberration is said to be totally abolished, and the spherical aberration materially modified, while the natural colors of the object examined are preserved. These objectives are usually made to order, and are only adapted for a particular length of draw-tube. The highest powers as yet in the market are the $\frac{1}{8}$ and $\frac{1}{12}$ immersion. Seven varieties of eye-pieces are made, with powers of magnification from 1 to 27.

From personal examination I can recommend these glasses most highly. The term apo-chromatic means merely that the objective is so corrected as to remove all color, and the term *compensation* as referring to an eye-piece means that the aberrations, especially the spherical, are remedied by the eye-piece rather than by the lens. As a result, he is able to use a wide-angled anterior lens, and obtains more working distance than usual.

Microscopic Appliances and Accessories —The following list embraces the most important appliances for a laboratory:

1. Glass bottles with ground stoppers for the alcoholic solutions of aniline dyes.

1. Glass bottles with glass funnels for the watery solutions of aniline dyes.

3. A number of watch-glasses of rather large size for staining sections, or gallipots with flat bottoms.

4. Glass slides and glass covers of the best quality, and as thin as can conveniently be used.

5. Holders for platinum needles and the ordinary milliner's needles.

6. One or more section lifters, of which there are many patterns.

7. Finely-pointed forceps, known as the iris forceps.

8. Collapsable boxes for Canada balsam.

9. Boxes for preparations. (All microscope-makers can furnish these in great variety.)

10. Labels for slides.

11. The immersion lens should be provided with a glass-stopped vial for the special immersion fluid that is used. Zeiss uses cedar oil.

The Microtome.—This instrument is now used in all laboratories, as it institutes great precision in the cutting of sections, and obviates waste of material. There are many kinds of microtome made, both abroad and at home. Unfortunately, they are still quite expensive. The instrument known as Schanz's*

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* These microtomes may be purchased from Meyrowitz Brothers, corner of 23d St. and 4th Ave., N. Y. City.

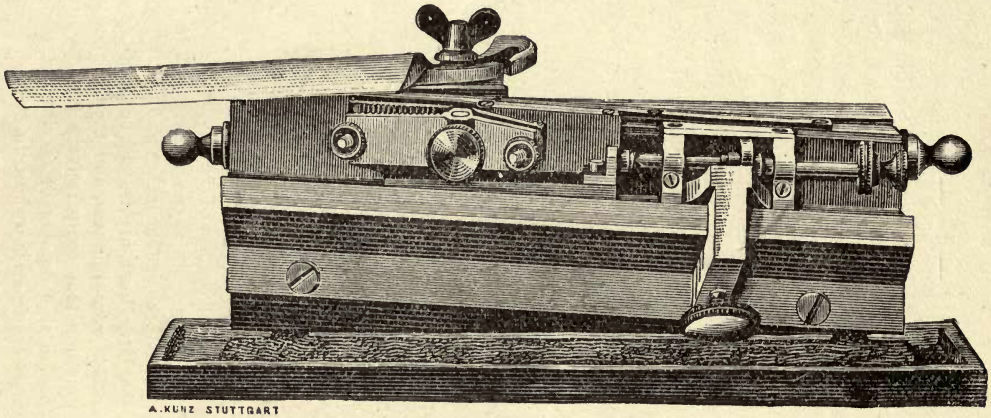

Fig. 2.—The Thoma Microtome.

is very highly esteemed, and so also is Thoma's*, but any one of the many can be made to operate successfully after the student has acquired a certain amount of familiarity with its use. It is best to have a microtome that has a freezing attachment, so that cuts may be made from fresh material.

Reagents.—The following are desirable:

1. Ordinary alcohol of 90 per cent. strength.
2. Absolute alcohol.
3. Oil of cloves or of bergamot for clarifying sections.
4. Celloidine to be used for mounting material for cutting in the microtome. Celloidine dissolves in equal parts of ether and alcohol, and should be kept in a wide-mouthed bottle.
5. Gelatine of good quality.
6. Acetic acid.
7. Aqua ammonia.
8. Glycerine.
9. Nitric acid.
10. Distilled water.

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* The principal advantage of the Thoma microtome is the uniformity of the sections which can be made with it. Sections of large size, up to three or four inches in diameter, which can be cut with the large instruments, will be found, as a rule, to be of a remarkably even thinness, and they are cut with such mechanical precision that comparatively few have to be discarded. Hence, a large number of excellent sections can be cut from a single specimen in a short time.

11. Sterilized water.

12. Ashphalt lac.

13. Canada balsam dissolved in zylol.

14. Acetate of potash, concentrated solutions, and the following:

Glycerine gum (Farrant's solution):

Glycerine.....
Water.....
Saturated solution of arsenious acid .....

Take of these equal parts; mix and add solution of gum arabic a half part.

Glycerine-gelatine (Klebs'). This is made in the following way:

Take of washed gelatine..... 10 parts.

Having added distilled water to the gelatine, pour off the excess of water, melt the gelatine, and then add:

Glycerine..... 10 parts.

Finally, add a few drops of carbolic acid to prevent moulding.

Acidulated alcohol:

Alcohol..... 10 parts.
Hydrochloric acid..... 1 part.

Aniline water:

Distilled water..... 100 parts.
Aniline..... 5 "

Muller's fluid:

Bichromate of potash..... 2 parts.
Sulphate of sodium..... 1 part.
Distilled water..... 100 parts.

Osmic acid:

Distilled water	100 parts.
Osmic acid	5 "

Osmic acid should be placed in a stoppered black bottle and protected from the light, and be put in a cool place.

Iodine solution:

Iodine	1 part.
Iodide of potassium	2 parts.
Distilled water	50 "

Alum-carmine:

Carmine	1 part.
Five-per-cent. solution of alum	100 parts.

Picro-carmine (Ranvier):

Carmine	1 part.
Distilled water	10 parts.
Solution of ammonia	3 "

Magenta solution (Gibbes'):

Magenta	2 parts.
Aniline oil	3 "
Alcohol (Sp. Gr. .830)	20 "
Distilled water	20 "

Gentian violet:

(*a*) Saturated alcoholic solution .....

(*b*) Aqueous solution.....

Gentian violet	2.25 parts.
Distilled water	100 "

Hæmatoxylon solution.

Eosine:

(*a*) Saturated alcoholic solution .....

(*b*) Aqueous solution.....

Distilled water ..... 100 parts.
Eosine ..... 5 "

Bismarck brown:

(*a*) Concentrated solution in equal parts of glycerine and water, or in water only.

(*b*) Aqueous solution.....

Bismarck brown ..... 2 parts.
Alcohol ..... 15 "
Distilled water..... 85 "

Ebner's solution. This consists of the following ingredients:

Hydrochloric acid..... 5 parts.
Alcohol ..... 100 "
Distilled water ..... 20 "
Chloride of sodium..... ... 5 "

Neelsen's solution:

Dissolve fuchsine ..... 1 part.
In a 5-per-cent. watery solution of carbolic acid..... 100 parts.
Add alcohol.... ..... 10 "

Gibbes' solution:

Rosaniline hydrochlorate ..... 2 parts.
Methylene blue..... 1 part.
Triturate in a glass mortar.
Dissolve aniline oil..... ..... 3 parts.
In rectified spirit..... 15 "
Add slowly to the above.
Slowly add distilled water..... 15 "
Then keep in stoppered bottle.

Fuchsine:

(*a*) Saturated alcoholic solution.

(*b*) Aqueo-alcoholic solution.

Fuchsine	2	parts.
Alcohol	15	"
Water	85	"

Methylene blue:

(*a*) Concentrated alcoholic solution.

(*b*) Aqueo-alcoholic solution.

Methylene blue	2	parts.
Alcohol	15	"
Water	85	"

Koch's solution:

Con. alcoholic solution of methylene blue	1	part.
Ten-per-cent. potash solution	2	parts.
Distilled water	200	"

Loeffler's solution:

Con. alcoholic solution of methelene blue	30	parts.
Solution of potash, 1–10,000	100	"

Methyl violet:

(*a*) Con. alcoholic solution.

(*b*) Aqueous solution.

Methyl violet	2.25	parts.
Distilled water	100	"

(*c*) Koch's solution.

Aniline water	100	parts.
Alcoholic sol. of meth. viol.	11	"
Absolute alcohol	10	"

Picric acid:

(*a*) Con. alcoholic solution.

(*b*) Saturated aqueous solution.

Kleinenberg's solution:

Saturated watery solution of picric acid	100	parts.
Strong sulphuric acid	2	"

Filter and add:

Distilled water	300	"

Orseille (Wedl):

Dissolve pure ammonia free orseille in absolute alcohol	20	parts.
Acetic acid	5	"
Distilled water	40	"

Until a dark red liquid results, then filter.

Safranine:

(*a*) Concent. alcoholic solution.

(*b*) Watery solution, 1-per-cent.

Vesuvine:

(*a*) Concent. alcoholic solution.

(*b*) Watery solution.

All the material, instruments, microscopes, etc., needed for bacteriological study, can be obtained from G. Koenig, Berlin, N. W. 35 Dorotheen Strasse, Germany. Chemicals, nutrient gelatine, etc., can be obtained from Dr. Georg Gruebler, Leipsig, 17 Dufour Strasse. He also furnishes Grenacher's borax-carmine, Weigert's picro-carmine, and Orth's picro-lithium-carmine. Many of these special reagents and appliances can be obtained from Meyrowitz Bros., 23d St. and 4th Ave., New York City.

SPECIAL APPLIANCES FOR STERILIZING.*

STERILIZING APPARATUS.

The Steam Sterilizer (Fig. 3).—This is a cylindrical vessel of tin, covered with a jacket of thick felt. A lid or cap similarly covered fits over the top. From its summit projects a thermometer. The interior of the vessel is provided with a wire grating, or a series of holes, to support the material to be sterilized. After the chamber has been fillled to the desired height with water, heat should be applied by one or more gas burners. The water should only reach to the point indicated upon the water-gauge. When the thermometer registers a temperature of 100° C., then the material to be sterilized should be introduced, but not before. The sterilizer is employed for sterilizing nutrient media, whether in boxes or flasks, for cooking potatoes, or for various other purposes, as will be seen.

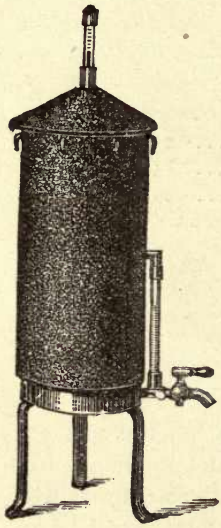


Fig. 3 Koch's Steam Sterilizer.

* All of the apparatus described in this section can be obtained from Messrs. Eimer & Amend, 205–211 Third Av., New York City. This firm is now prepared to furnish the full equipment for a bacteriological laboratory and manufacture any new apparatus according to the most recent models.

The Double-walled Hot-air Steri'izer.—This is a square tin box with double walls, and designed either to stand upon four legs, or to be suspended from the wall of the laboratory. (Fig. 4.) It is heated in the ordinary way by a Bunsen's compound or rose

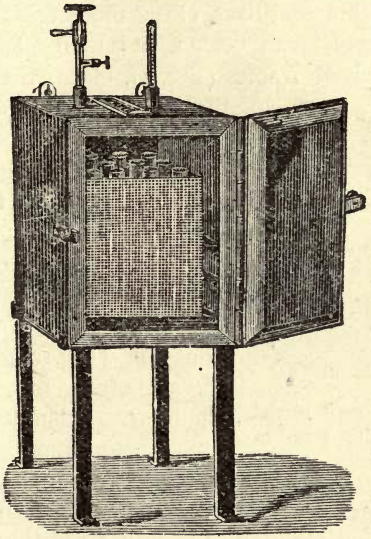
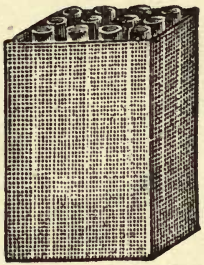


Fig. 4. Double-walled Hot air Sterilizer.

burner, and the internal temperature is indicated by a thermometer introduced through an opening in the top. In a second opening a gas-regulator can be placed. The apparatus is used for sterilizing at

higher temperatures than are practicable by the steam sterilizing method first described. It is specially adapted for sterilizing the vessels designed for receiving the material used for purposes of study, such as the receivers for blood serum, glass flasks, glass plates for nutrient media, accessory appliances, etc. In using this sterilizer, close the sterilization tubes with a plug of clean cotton, and then place them in the galvanized frame (Fig. 5), place the frame in the sterilizer and heat for one hour to 140° C. It will be found that this degree of heat will slightly char the cotton plug. When glass plates are used for the study of water, as will be seen at another place, they are to be put in a covered iron box (Fig. 6), and heated, as before, to 140° C. for one hour. Erlenmeyer's flasks which are large glass flasks used as storage vessles for nutrient media, whether containing gelatine or agar-agar, are not placed in the galvanized basket, but directly in the sterilizer.



The basket-rack for receiving the sterilization tubes.

The Water Bath.—This is the ordinary water bath that is used in chemical laboratories.

The Hot-water Filter.—This is a heavy copper funnel having double walls, the space between them

being filled with hot water. A glass funnel is placed in the copper funnel, and its stem, which is made to project beyond the stem of the copper funnel, is held

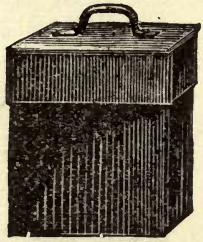


Fig. 6. The metal box for glass plates that are to be sterilized.

by a rubber plug that fits in the copper stem. The water in the copper funnel is heated by a gas flame applied to a lateral projection of the funnel. It stands upon three legs. (Fig. 7.)

There is another and more recent model of hot-water filter, in which the copper and glass stems are longer than in the filter just described, the heat being applied to the funnel by a circular burner, which at the same time serves as a funnel-ring. There should also be on hand a goodly supply of *glass funnels* of various sizes, *test-tubes* and *flasks*, cylindrical *graduates* marked off in cubic centimeters, aud a good *balance* with gram weights. The *gelatine* for use should be of the best quality, and the "gold label" is generally preferred. As gelatine is not suitable for use

when the temperature of the atmosphere is high, *agar-agar*, or Japanese isinglass is used. It consists of the filaments of certain algæ. There should also be on hand: *Table salt*, *litmus papers*, *filter paper* of various descriptions, *flannel* for filtration purposes,

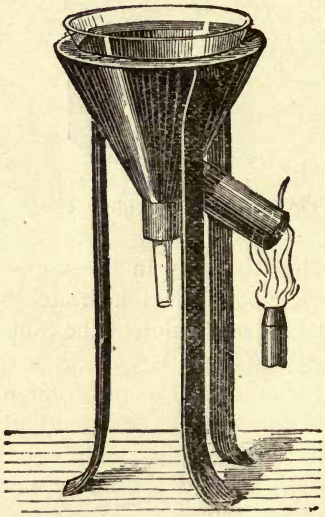


Fig. 7. Hot-water Filter.

lactic acid, and *carbonate of soda* in saturated solution. There will also be use for *anatomical jars* with wide mouths, and *test-tube stands*. *Rubber caps* for covering the cotton plugs are sometimes needed. The platinum needle that is used for inoculation purposes consists

of a platinum wire several inches in length, embedded in a glass rod. Some needles are straight, others are curved or looped. The latter is called in Germany an *oese*. (Fig. 8.)

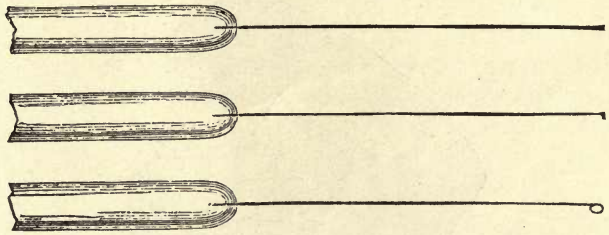


Fig. 8.—Platinum Needles.

It is necessary to have a number of *shallow glass covers* (Fig. 9) and dishes to serve as damp chambers in which the glass plates remain while the process of cultivation proceeds in the nutrient media poured upon them. The *glass plates* are usually 4x6 inches in superficial measurement.

Glass benches are shelves made of glass to support glass plates or slides in tiers, when they are placed in the glass chambers. They are easily made by cementing glass plates and slips together, (Fig. 10 & 11) but the best are made of but a single piece of glass.

Glass rods should be on hand in considerable quantity. They are used for spreading the liquified agar-agar or gelatine on the glass plates.

APPARATUS FOR PREPARING POTATOE CULTIVATIONS.

In order to sterilize the instruments used in preparing potatoes for cultivation, it is customary to have a small iron case of simple construction, to contain

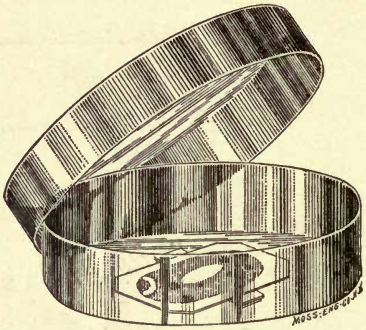


Fig. 9.—Glass dishes with glass covers forming a moist chamber, in which a glass plate covered with nutrient gelatine lies upon a single bench. The dark areas in the gelatine correspond with different cultures of bacteria.

a few scalpels and other knives for cutting potatoes. The box is then sterilized, together with its contents, by exposure to dry heat in the hot-air sterilizer (Fig.

Fig. 10. Glass Benches.

Fig. 11.

3) for one hour, at 140°–150° C. The instruments for such a case need no special description.

The damp chambers in which the potatoes are cultivated is the same in design as the ordinary damp chamber used for plate cultivations (see Fig. 9), though they need not be so large.

APPARATUS FOR THE PREPARATION OF SOLID STERILE SERUM.

First of all, it is necessary to provide one's self with a *glass jar*, Fig. 12, with wide mouth and glass

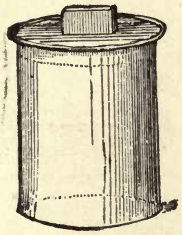


Fig. 12.—Glass Jar for holding Blood.

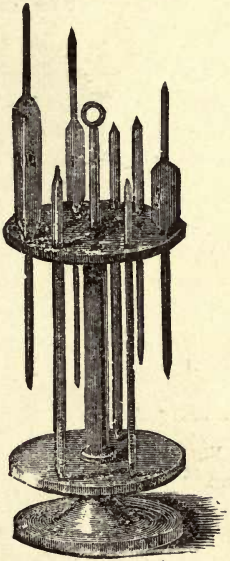


Fig. 13.—Rack for holding Pipettes.

stopper, for securing the blood. Then there should be *pipettes* (see Fig. 13), graduated or not, as the case may be, for transferring the serum from the jar to sterilized vessels.

Koch's Steam Sterilizing Apparatus for Blood Serum (Fig. 14) consists of a cylindrical vessel,

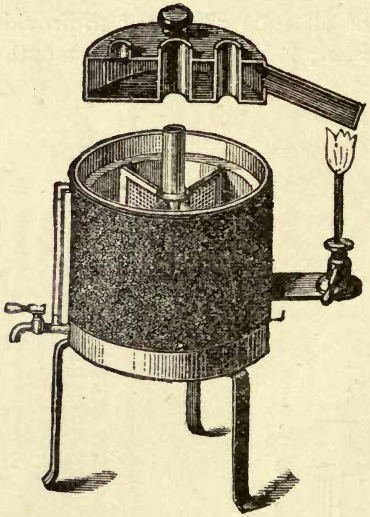


Fig. 14. Koch's Steam Sterilizing Apparatus for Blood Serum.

double-walled, covered with felt and fitted with stop-cock or steam guage. The space between the walls is designed to hold water, and a felt-covered lid pro-

vided with a tubular prolongation is connected with the water chamber. The water in the border of the vessel is heated by a gas flame from below. The water in the lid is heated by a flame applied to the prolongation.

In the centre of the cylinder is a column which communicates with the water chamber of the cylinder, and from it pass four partitions, which serve to support the test-tubes.

In the lid are three openings, one of which communicates with the water chamber in the lid by which the latter is filled and into which a thermometer is then fixed. In the centre an opening admits a thermometer, which passes into the central pipe of the cylinder. Through a third opening a thermometer passes to the cavity of the cylinder.

The apparatus is supported on legs. The method of procedure is as follows: Fill both body and cover of the apparatus with water, being careful to keep the inner space perfectly dry. Use two burners and raise the temperature to 60° C. The blood serum which has previously been placed in the sterilization flasks (already sterilized by apparatus No. 4) is now deposited in the hollow space of the apparatus, and the cover is replaced. The temperature is now raised to 58° C. The serum should remain liquid, and, therefore, the temperature is not to be raised above 60° C. In order to solidify the serum, a special apparatus has been devised by Koch (Fig. 15). It consists of a

rather shallow tin box, with glass cover, and stands on four legs. The whole is jacketed with felt. Like some of the other vessels already described, it is double-walled, and the water is heated from below by the flame of a gas burner. Of the four legs the front ones rest in grooves of the box, and slide up and down so that the box can be depressed in front to a limited extent. The box is filled with from 50 to 100

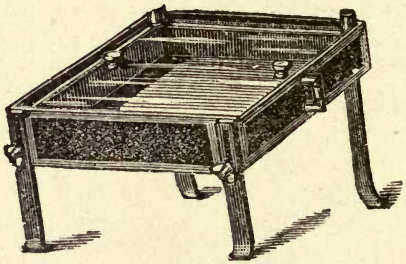


Fig. 15.—Apparatus for Solidifying Blood Serum.

tubes of a nutrient media, such as serum, gelatine, or agar-agar, and it is intended, by inclining the front of the box, to give a sloping surface to the nutrient media. To use the apparatus, place the tubes containing the nutrient, after sterilization, in the apparatus, inclining each tube at the desired angle; then heat to 65° or 75° C. The serum should then become a transparent fluid, and so remain, and the heat should be applied until this result is attained. The small quantity of water which forms is allowed to remain.

This is called the *water of condensation*, and has an important office. *Glass capsules* are small cubes of crystal glass, hollowed out. They are used for cultivations in solid serum, gelatine, or agar-agar. They are made of white or colored glass, and are fitted with glass covers.

APPARATUS FOR STORING, AND FOR CULTIVATING IN LIQUID MEDIA.

Lister's Flasks.—These are globe-shaped flasks with two necks—a vertical and a lateral one. The lateral one is a bent spout tapering towards the extremity. A drop of liquid remains behind in the end of the nozzle, which prevents regurgitation of air through the spout when the vessel is restored to the erect position, after some of its contents have been poured out. A cap of cotton wool is placed over the orifice, and the residue kept for further use. The vertical neck of the flask is plugged with sterilized cotton wool, in the ordinary way.

Sternberg's Bulbs.—These are bulbs provided with slender necks drawn out to fine points, and hermetically sealed.

Aitken's Test-tube.—This has been advised for counteracting the danger of the entrance of atmospheric germs on removal from the ordinary test-tube of the cotton wool plug. Each test-tube has a lateral arm tapering to a fine point, which is hermetically sealed.

APPARATUS FOR INCUBATION.

There are several kinds of incubator; most of them are rectangular chests with glass walls front and back, or in front only. A cylindrical model is preferred by some. The two described here are those of D'Arsonval and Babes. The former admits of very exact regulation of temperature, while the latter is a very practical form for general use.

D'Arsonval's Incubator.—The "Étuve D'Arsonval" (Fig. 16) is a very efficient apparatus, and is provided with a heat regulator, which enables the temperature to be maintained with a minimal variation. It consists of a cylindrical copper vessel with double walls enclosing a wide inter-space for containing a large volume of water. The roof of the water-chamber is oblique, so that the wall rises higher on one side than on the other. This admits of the inter-space being completely filled with water. At the highest point is an opening fitted with a perforated rubber stopper, through which a glass tube passes. The mouth of the cylinder itself is horizontal, and is closed by a lid, which is also double-walled to contain water. In the lid are four openings; one serves for filling its water-chamber, and the others for thermometers and for regulating the air supply in the cavity of the cylinder. The cylinder is continued below by a cone, also double-walled, and there is a perforated grating at the line of junction of the cylinder and cone. The cone terminates in a projecting tube provided with an

adjustable ventilator. The apparatus is fixed on three supports, united to one another below. One of them

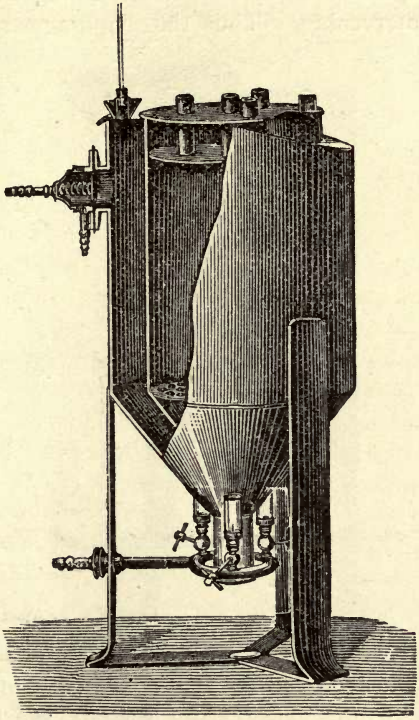


Fig. 16.—D'Arsonval's Incubator.

is utilized for adjusting the height of the heating ap-apparatus (Fig. 16), attached to a circular-lipped

aperture in the outer wall of the incubator. To the lip is fixed with screws the corresponding lip of a brass box, with a tightly stretched diaphragm of india-rubber intervening. Thus the diaphragm separates

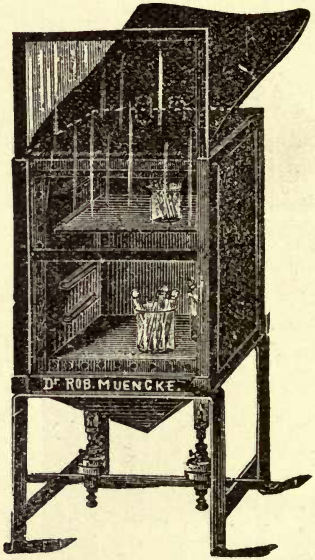


Fig. 17.—Babes' Incubator.

the cavity of the box from the water in the inter-space of the incubator. The cap of the box, which screws on, is bored in the centre for the screw-pipe by which the gas is supplied. Another pipe entering the box from below is connected with the gas-burners. Around

the end of the screw-pipe a collar loosely fits and is pressed against the diaphragm by means of a spiral wire spring. Close to the mouth of the screw-pipe a small opening exists, so that the gas supply to the burners is not entirely cut off even when the diaphragm completely occludes the mouth of the screw-pipe.

Babes' Incubator.—The pattern of Dr. Babes' is very simple, and is now used in most laboratories. (Fig. 17.)

It consists of a double-walled chest with sides and roof jacketed with felt. Water fills the interspace between the walls, and on the roof are two apertures, one for a gas regulator and the other for a thermometer. In front the chest is closed in by a sheet of felt, a glass door, and a sliding panel. The apparatus can be suspended on the wall or supported on legs, and is heated from below by means of protected burners. The gas should pass through a thermo-regulator to the burners. Reichert's thermo-regulator (Fig. 18) is very generally used and it will maintain the heat at a very equable temperature. It consists of a vertical glass tube (Fig. 18 *c*) with a rectangular arm fitted with a pressure screw (*s*)

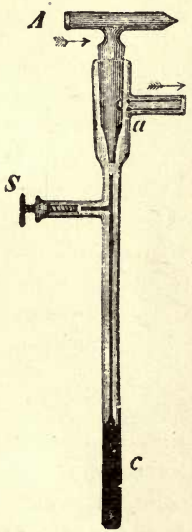


Fig. 18.—Reichert's Thermo-regulator.

and another rectangular arm (*b*). The gas enters by the tube (*a*) which is inserted in the tube already described. As the heat expands the mercury in the vertical tube (*c*), the column rises and diminishes the volume of gas entering; as the column falls more gas again enters, and so as the column rises and falls the supply of gas is increased or diminished.

The pressure-screw (*s*) also assists in securing the precise amount of heat that is necessary, by the pressure it makes upon the column of mercury.

GENERAL LABORATORY REQUISITES.

Siphon Appa·atus.—Two halfgallon or gallon bottles with siphons connected with long flexible tubes provided with glass nozzles and pinchcocks should be employed for the following purposes: One is used to contain distilled water, with the nozzle hanging down conveniently within reach of the working table; the other should contain a solution of corrosive sublimate (1 in 1000), and may be placed so that the nozzle hangs close to the sink or basin. The former is used instead of the ordinary wash bottle, and the latter for disinfecting vessels and hands.

Dessicator.—The dessicator consists of a porcelain pan containing sulphuric acid; it is covered over with a bell-glass receiver. In the centre of the pan is a column supporting a circular frame, which is covered with wire gauze. Slices of potatoes upon which micro-organisms have been cultivated, are

rapidly dried by the action of the sulphuric acid in confined air.

Besides the items already described, there are many others in use, such as air-pumps, refrigerators, etc. These are used for special investigations, and too numerous to detail here.

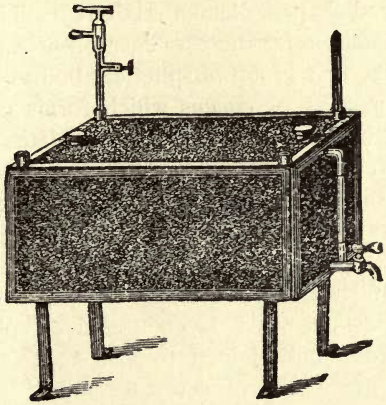


Fig. 19.—A Simple Incubator or Brood Oven.

In Fig. 18 is seen another and simple form of incubator. It is lined with lead, covered with glass and felt, is fitted with water-guage and stop-cock and has openings for a thermometer and gas regulator.

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* Most of the matter in this section has been taken freely from Crookshank.

CLASSIFICATION OF BACTERIA.

Bacteria, called also schizomycetes, or fission fungi, have been classified in various ways. The classification of Zopf is now in high estimation, but none is destined to live for any great length of time. Zopf divides them into four groups, viz: the coccacceæ, bacteriaceæ, leptotricheæ, and cladotricheæ, *i. e.*, the spherical, rod-shaped, filamentous, or branching micro-cymes. Some prefer the classification of Fluegge.

1. The first group of spherical bodies comprises the streptococcus, or coccus which forms chains, the merismopedia, or coccus that forms plates or lamellæ; the sarcina, or packet coccus that forms colonies in cubes; the micrococcus that remain in aggregations of irregular form; the ascococcus that form gelatinous pellicles.

2. Under the group bacteriaceæ is found the genus bacterium, which is composed of spheres and rods, or rods only, joined to firm rods; the genus spirillum, made out of screw-formed threads; the bacillus; the vibrio, which is shaped like a pin, having a nodular extremity.

3. The third group, leptotricheæ, comprises four genera. Most of them are found in water. An example is the leptothrix buccalis.

4. Of the last group, the cladotricheæ, only one example, the cladothrexi Foersteri, occurs in the human being; it is found in the lachrymal canal of the human eye.

CHAPTER III.

METHODS OF EXAMINATION.

The new methods for the detection of micro-organisms depend mainly for their success on improved methods of illuminating and coloring the objects. To obtain the first we now use the immersion objectives with special condensers, which have been described, and also aniline colors, of which the variety is infinite. These organisms, which come under the general name of bacteria, may be present in the solid or fluid tissues of the body, and it may be necessary to examine them in these media; or we may have to study them in certain fluids, called *cultivation fluids;* or thirdly, they may have to be examined as they grow upon certain solid substances, such as gelatine, potatoes, blood-serum, etc.

Most of these organisms have an extraordinary resistance to various chemical reagents, and it is by this means often that we distinguish them from other substances of similar forms. Thus, they are not acted upon by acids which usually destroy most inorganic granules; nor are they affected by ether, alcohol, or chloroform, which destroy fatty granules and even crystals.

In bacteriological work one of the most essential considerations is cleanliness. All of the reagents, instruments, etc., should be cleansed before using.

This is especially necessary for all slides and cover-glasses, and it is even necessary, somtimes, to leave these latter in strong acids, sulphuric, nitric, etc., for some hours, after which they are to be washed and carefully cleansed. So also in the employment of cultivating media, strict attention should be paid to details, or else the work will be unsatisfactory.

Examination of Fresh Substances.—To examine liquids containing bacteria, a drop of the substance, such as blood, pus and the like, is to be transferred with a *sterilized pipette*, or *loop*, to a slide, and then covered with a clean cover-glass. Fresh tissues may be teased with needles in a sterilized salt solution, and then mounted between the slide and cover-glass. Cultures on solid media, such as potatoes and the like, can be examined in the same way, a small portion being transferred with a sterilized needle to a drop of sterilized water on a slide.

Another method is as follows: Take a thin cover-glass, draw it across the cut surface of the tissue or the liquid, then taking another cover-glass, squeeze the two together, then separating them; each one is found to be covered with a thin layer of the material to be examined. They are then dried; afterwards they are seized by the forceps and passed rapidly through the flame of a Bunsen burner, with the specimen on the upper side of the glass. If it be desirable to stain them, drop two or three minims of your staining fluid on the film; then, after a minute or two,

wash off the surplus stain with distilled water by means of a wash bottle, place your cover-glass on a slide, soak up the excess of water with filter-paper, and then examine under the microscope. If such a specimen is to be preserved permanently, dry and mount in Canada balsam in the usual way.

Baumgarten's Method.—Place a drop or two of the fluid to be examined on a cover-glass that has been thoroughly cleansed; press the fluid into a thin layer with another cover-glass, as has already been described. After the covers have been dried and passed three times through the flame of a Bunsen burner, immerse the covers in a solution of two drops of a 33-per-cent. solution of caustic potash to the watch-glass of water; then mount the cover-glass on a slide and examine with a high power. The bacteria will then be readily seen as bright shining bodies. To prepare specimens that have been hardened in alcohol, or fresh specimens, proceed as follows: Place the sections first in absolute alcohol for several minutes, then immerse in ether or alcohol, and then in a strong solution of acetic acid, then wash in distilled water, and afterwards warm in a 2-per-cent. solution of caustic potash. The potash dissolves the fat granules and the small crystals, and destroys the tissue, leaving only the bacterial bodies for examination.

Ehrlich's Method.—This is as follows: Take 5 parts of aniline oil, add 100 parts of distilled water, then filter the emulsion carefully. To the filtrate, in a

glass, add drop by drop an alcoholic solution of fuchsine, methyl violet, or gentian violet. Cover-glass preparations should be immersed in this liquid at least half an hour; they are then to be washed for a few seconds in dilute nitric acid, 1 part to 2 of water, and then in distilled water. Everything now, except the bacillus, is removed.

Babes' Method.—This is a rapid method for examining cultures. A small portion of the growth removed by a sterilized hook is smeared on a cover-glass so as to form a thin film; after it becomes dry a few drops of an aqueous solution of methyl violet are let fall upon the film; the excess of color is removed by a strip of filter paper.

His's Method.—This is also suited for fresh specimens. A slide is prepared as has already been described, but the reagents are applied by a pipette at one edge of the cover-glass, and withdrawn from beneath the cover by means of a strip of filter paper placed at the opposite edge.

Cover-glass Impressions.—A perfectly clean cover-glass is placed on a plate or potato culture and gently pressed into it; it is then carefully raised by forceps and allowed to dry after being passed through the flame three times. The growth in this way is bodily transferred to the cover-glass.

Gram's Method.—Specimens prepared by this method should be kept in absolute alcohol, from which they are transferred at once to Weigert's or Ehrlich's,

gentian violet, or fuchsine and aniline water solutions, where they remain for several minutes, though tubercle sections should be kept at least twenty-four hours, afterwards washed for three minutes in alcohol, and then in a solution of 10 parts of iodine, 20 parts of iodide of potassium, and 3000 parts of water, until a dark blue violet is changed to a dark purple red. Now wash in alcohol until most of the color has disappeared, then clarify in oil of cloves. Such sections are to be mounted at once in balsam, when the tissues will be seen to have a faint yellow color, and the bacteria are deep blue or black. Most micro-organisms can be stained by this method, but some require a different treatment. For example, the bacillus of glanders is best stained with an alkaline solution of methyl blue, which is prepared (Schutz) by making a one-tenth part per thousand watery solution of caustic potash and adding one-third the bulk of a saturated solution of methyl blue. These specimens are then treated with dilute acetic acid, and mounted in the regular way. So also the typhoid bacilli are said to be stained best by this method—not so well by Gram's method. For certain organisms, such as the micrococcus of pneumonia in the sputum, and the micrococcus of gonorrhœa, Klein recommends a mixture of methyl blue and vesuvin.

Friedlaender employes the following method for the micrococcus of pneumonia: He takes the solution of fuchsine, 1 part; distilled water, 100 parts; alcohol

and glacial acetic acid, 2 parts; immerses the sections in it, then washes them in alcohol, next in a 2-per-cent. solution of acetic acid, then in alcohol and oil of cloves, and mounts in balsam.

To Stain Tubercular Bacilli. — The tubercle bacillus takes the following dyes slowly, but holds them steadily:

The method of Ehrlich is recommended by Koch.

Kaatser's Method.—After staining the film on the cover-glass with aniline water and alcohol gentian-violet solution, he immerses them from one-half to one minute in a solution of alcohol, 90 per cent., 150 parts; distilled water, 30 parts; hydrochloric acid, 1 part; he then washes them thoroughly from one to two minutes in 90-per-cent. alcohol, until the coloring matter disappears from the film; he then dries and adds a watery solution of vesuvine. After a couple of minutes he again washes in distilled water, dries, and mounts in balsam.

Gibbes' Method.—This may be used, especially where a double staining is desirable. He takes of rose-aniline hydrochloride, 2 parts; methyl blue, 1 part; triturates in a glass mortar, then he dissolves aniline oil, 3 parts; in rectified spirits, 15 parts. He then drops the cover-glass with hardened sputum on it in this solution after it has been warmed, and keeps it there for four or five minutes. He then washes it in alcohol until no more color comes away, after which he mounts it in Canada balsam.

Weigert's Method.—For staining solid tissue containing bacteria, place the sections from six to eighteen hours in a 1 per-cent. watery solution in any of the aniline dyes. If it be desirable to hasten the process, place the sections in an incubator and heat to 45° C. If a stronger solution be used, the sections may be over-stained. They are then treated with carbonate of potash in half-saturated solution. Next the sections are washed with distilled water and passed through 60-per-cent. alcohol into absolute alcohol. when almost decolorized, lift the sections from the fluid, dry, and transfer to oil of cloves; preserve in Canada balsam.

To Harden and Decalcify Preparations.—In hardening small organs, place on a piece of filter-paper at the bottom of a glass jar, and cover with twenty times the volume of absolute alcohol; or, Mueller's fluid may be used for a day or two, and then absolute alcohol. Tissues prepared in this way are ready for cutting in two or three days. Teeth or bones must be placed in a decalcifying solution, such as Kleinenberg's; when sufficiently softened they should be soaked in water to wash out the picric acid, then transferred to alcohol.

To Imbed and Cut Specimens.—Material to be cut with a freezing microtome should be soaked in water before being frozen. In some instances the hardened tissues should be well soaked first in gum mucilage and then frozen. In other microtomes, the tis-

sues are imbedded in paraffine or celloidine, and mounted on cork; celloidine is more commonly employed. The tissues to be imbedded are placed in a mixture of ether or alcohol for an hour or more; they are then transferred to a solution of celloidine in equal parts of ether and alcohol, and left there for several hours. Corks ready for the clamps of the microtome are covered over with a solution of celloidine, then this is applied with a glass rod to the surface which is to receive the tissue. The tissue remains in celloidine solution from one to twenty-four hours. In some tissues, as in the lung, a longer time is necessary. A little of the solution which is of a syrupy consistence, should be allowed to fall on the tissues so as to cover them completely, and the mounted specimen is placed in a 60- or 80-per-cent. alcohol to harden the celloidine. The specimen will be ready for cutting next day. The celloidine in the sections disappears during the process of clarifying in clove-oil.

CHAPTER IV.

CULTURE METHODS.

Dr. Koch, of Berlin, has, as I have already shown, introduced improvements in the investigation of bacteria by means of the process of cultivation, which Pasteur originated. By it the special bacteria, which are the cause of any particular change in the tissues or fluids, may be eliminated, so that forms which are harmless may readily be separated from those that are poisonous. This process, which seems difficult, can readily and successfully be accomplished if proper care be exercised. If, for example, we wish to select the bacteria which give rise to a peculiar coloration in a liquid, we take a small quantity of the liquid, just enough to cover the point of a needle, and then mix it with about a teaspoonful of gelatine, which becomes fluid at 35° C.; for the bacterial organisms which have been taken up by the needle are disseminated through the melted gelatine, which is then spread upon the flat surface, excluded from the air, and after a variable period, usually from one to three or four days, we obtain from each individual spore a distinct culture, some of one color, some of another; these are *pure cultivations.* The next step consists in taking from each of these fields and sowing again in fresh melted gelatine. If now we wish to prove to ourselves that we have discovered the special parasite, we take

this special liquid, free from bacteria, and inoculate it with the colored patch that we suspect is the cause of the coloration. If the same color be produced as in the original instance, there is *prima facie* evidence that we have discovered the special parasite. This is the general plan under which Koch proceeded to eliminate the common bacillus of cholera and the organisms that he claims are the causes of pyæmia, pneumonia, etc. This method of gelatine culture is simple enough to be practiced by any intelligent observer. It has come to be held now-a-days, that the color or the naked-eye appearance of the growth of a culture rather than its morphological character, determines the relationship of a micro-organism, for the form of these bodies is very changeable, each species being subject to alterations in form, according to the menstruum in which it is found. To have a culture successful, the medium must necessarily be completely sterile; but this may be either liquid or solid, some micro-organisms growing better in one than in the other. Thus, it is said that the bacteria of chicken cholera will die within 48 hours if immersed in a decoction of beer-yeast, which is an excellent nutrient fluid for putrefactive bacteria.

Fluid Media.—A common nutrient medium is a meat decoction of any kind. It should be free from oily matter, of neutral reaction, free from particles of any kind, and sterile. Vegetable decoctions and infusions are also used, but less frequently.

The method of sterilizing a culture medium is chiefly by subjecting it to a temperature sufficient to kill any form of bacterium. This may be accomplished in several ways.

Pasteur's Method consists in placing the culture fluid in small pear-shaped flasks, which are blown from a glass tube and then hermetically sealed. These flasks are then bathed in a solution of chloride of lime or nitrate of soda, and kept there for twelve hours at a temperature of 110° to 115° C.

Buchner's Method.—A kettle 27 centimetres in diameter and 45 centimetres deep is used; this can be closed steam-tight. Water is then poured in until it is 5 to 8 centimetres deep. The test-tubes are arranged in tiers, and then suspended one above the other in the kettle; each tube is closed with cotton and covered with cloth at the top. After the cover of the vessel has been screwed on tight, the whole apparatus is heated for one hour and a quarter, and then kept for one hour at 110° C.

Miquel's Method consists in sterilizing by the use of filters. For the larger forms he uses porous paper; for the more minute, clay or plaster of Paris cups. He uses flasks, in the side of which, near the neck, is a small ventilation tube. The neck is also somewhat narrowed at its lower extremity. Now the mixture of plaster-of-Paris and asbestos is poured into the neck of the flask and allowed to dry slowly. The flask is then heated until the air has been expelled and the

bacteria have been killed. The ventilation tube is then fused together, and the culture fluid is poured upon the plaster plug in the flask, through which it is gradually forced. This method is used for the cultivation fluids that contain albumen, which would be coagulated by most of the methods that have been described.

Loeffler's Method.—This is the most popular now in use. The apparatus has already been described. This consists of a cylinder about half a metre high, and 20 to 25 centimeters in diameter; it has a copper bottom and is protected by a felt covering. At the lower third of the interior is a grate, and the space beneath is filled three-quarters full of water. The heat is applied by three or four gas flames; the lid of the cylinder is covered with felt. A thermometer passes through this lid; it has the advantage of not permitting the temperature to rise above 100° C.

SOLID OR GELATINE CULTURE MEDIA.

Solid Nutrient Media.—These are made by taking any of the fluid media just described, and adding to them pure gelatine or agar-agar, a material that becomes fluid at a low temperature, viz., 30° C. The advantage of solid media is that they enable the germs to be distributed widely, so that each germ can go on developing apart from its neighbor, and thus grow so as to form a considerable field visible to the naked eye. These solid media are better nutrient material

than the surface of a potato, and, indeed, it is difficult to separate the organisms of infective diseases from the putrefactive bacteria by growth on potato, because the latter grow so rapidly and develop so extensively that they soon conceal the former. When, however, pure cultivations have been obtained, they may, in some instances, be made to grow successfully upon potato. When a test-tube is used for solid cultures, the tube should be allowed to incline at an angle of 45 degrees, so that the surface of the gelatine will be larger. The objection to gelatine cultures is that they will not remain solid at the temperature best adapted for the growth of bacteria, namely, 30° to 37°; accordingly, Koch has devised another method.

Serum Culture.—The serum taken from the blood of an ox or sheep after the clot has been removed, is placed in test-tubes closed with cotton and rubber cloth, so that they are water-tight. They are heated one hour, daily, for six days, at 58° C., by which the serum is usually sterilized. It is then heated at 65° C., until the cotton is charred slightly. The serum now appears as yellow-colored, transparent, faintly opalescent, and firm. It should permit no bacteria to develop for several days. Material to be investigated is now placed upon this firm blood-serum, and the whole is kept at 37° or 38° C., until growth takes place —in twelve to fourteen days. Hydrocele fluid may also be used.

The Culture Vessels.—The common test-tube

closed with cotton is ordinarily used, but in some instances other forms are recommended. Some operators take a conical flask of glass having a flat bottom. They then employ a glass tube of a diameter sufficient to enter the neck of the flask, and somewhat longer than it. The tube is filled three-quarters full of asbestos wool, upon which is placed a pad of cotton. It is lowered rapidly in some cotton batting and pressed firmly into the neck of the flask. This apparatus, when heated to 200° C., is thoroughly sterilized.

Salmon's Tube.—The culture-tube of Salmon consists of a vessel like a test-tube with heavy glass, about five inches in length, and three-quarters of an inch in diameter. Over the top of this tube a hollow cap is fitted, and the edges so ground as to fit snugly over the surface of the reservoir, which is also ground. This cap, about two and one-half inches long, contracts near its middle into a narrow tube, about three-eighths of an inch in its internal measurement. A third tube, for ventilation, is like an inclined U, one limb about three inches long, and one and one-half inches longer than the limb which fits over the narrow tube. The short, free limb of the ventilation tube contains a plug of wool one and one-half to two inches long. The limbs of the ventilation-tube are about one inch apart. The culture fluid is introduced by removing the cap. The pipette used to introduce the fluid containing bacteria is an ordinary glass tube,

about one and one-quarter inches in diameter, and two or three inches long, drawn out into a fine, almost capillary tube, which should reach the bottom of the reservoir when introduced through the narrow tube of the cap; a cap of wool occupies the other end, which is closed by a rubber ball. The method of using it is as follows:

The pipette is first sterilized by super-heating every portion of it from the top of the capillary tube to near the rubber ball, until the air has been subjected to a temperature of about 150° C. It should be brought to a dull red heat. When cool, the capillary portion should again be drawn once or twice through the flame. The ventilator of the culture tube containing the bacteria to be sown is flamed and removed, and the narrow tube of the cap super-heated; the rubber bulb is slightly compressed and the pipette introduced, a few drops drawn up, the pipette withdrawn, the cap again super-heated, and the ventilator replaced. The cap of the fresh tube is now superheated before and after removing the ventilator; the pipette introduced, a drop allowed to draw into the the culture fluid, the pipette removed, the narrow tube of the cap again flamed, and the ventilator replaced. This method dispenses with cotton plugs and is easily learned. The tubes do not break easily.

Sternberg's Flasks.—These are easily made from glass tubing. They are little bulbs blown from a glass tube, and have a long neck that tapers gradually to a

capillary point. Each flask contains a sufficient amount of nutrient fluid and oxygen to insure a vigorous growth of organisms. When properly sterilized, the medium remains closed indefinitely, and the flasks may be packed away in drawers or boxes for use, or carried about from place to place. The inoculation from one flask to another, or from fluid, is easily accomplished with perfect security, or small amounts of fluid may at any time be withdrawn for microscopic examination without the danger of introducing foreign organisms, or, indeed, these flasks may be used as syringes, the contents being forced beneath the skin of a living animal if only gentle heat be applied to the ball, causing the air to extend and forcing the contents through the neck of the flask.

To Introduce a Sterilized Culture Fluid into a Sterilized Culture Vessel.—For this purpose a sharp canula is needed, made of silver, platinum, or glass, which is fastened with a rubber tube into the vessel containing the culture fluid. We previously sterilize the canula by holding it in steam escaping from the vessel, or by heating it in the alcohol flame. Remove the cotton plug from the vessel and push the canula through the asbestos into the vessel; now relax the clamp on the rubber tube and the culture fluid will flow over into the culture vessel. When it is not necessary to exercise great precaution against contamination, as in the pigment bacteria, cultures may be carried on under bell-glasses by sowing bacteria upon

solids, potatoes, turnips, or eggs, or upon gelatine or agar-agar upon glass slides.

Inoculation Experiments.—It is a matter of fact that certain animals only are susceptible to certain bacteria. For example, birds are not affected by anthrax bacteria. Then again, the mucous surfaces of the mammals are not suitable places for the introduction of germs, because they are already inhabited by myriads of bacteria. There are, however, two plans for the inoculation of pure cultures. The first is as follows:

Having prepared the skin by the removal of hair, or feathers, as the case may be, and having washed it with an antiseptic solution, dried it with absorbent cotton, and cut it with a sterilized knife, the bacteria of a pure culture are introduced by the sterilized flat wire, covering the spot with surgeons' antiseptic gauze, all contamination from the air is avoided; or, the material may be injected with a hypodermic syringe properly sterilized.

So far as our present experience goes, it cannot be said, however, that inoculation as yet has produced very positive results. Certain it is that inoculation for anthrax and pleuro-pneumonia in cattle has not stayed the progress of the disease in countries where it has been tried. Whether it will be a success in the future is a matter that remains to be determined.

CHAPTER V.

METHODS OF INVESTIGATING SPECIAL BACTERIA.

ANTHRAX.

Toussaint's Method.—In the year 1879, Professor Toussaint announced* that he could inoculate animals with the virus of anthrax, prepared in a peculiar way, and give entire immunity against subsequent disease. His plan was as follows:

First.—After defibrinating the blood of an animal suffering from anthrax, he subjected it to a temperature of 55° C. for about ten minutes. This was then inoculated upon a healthy animal.

Second.—He added to the anthrax blood one-fourth of one-per-cent. of carbolic acid, and then inoculated the carbolized blood.

According to his statements the bacteria were killed, but the infective material was not destroyed, but was rendered less virulent. This method was subsequently proven to be fallacious.

Pasteur's Method.—On the 28th of February, 1881, Pasteur, of Paris, announced to the Academy that he had discovered another method of successfully inoculating against anthrax. His method was simply to expose the anthrax poison to atmospheric air under

* Comptes Rend. Tom., 87, p. 1217, 1879.

certain conditions. He further stated that the anthrax bacteria reached their highest development at a temperature between 25° and 40° C.; at either higher or lower temperatures they increased or developed more slowly, and ceased to develop entirely at a temperature below 15° or above 45° C.

Pasteur found, as he stated, that the anthrax bacilli could be cultivated at a temperature between 42° and 43° C. in beef broth fluid; but when, under such circumstances, the fluid was exposed to filtered atmospheric air, the bacilli lost their virulence, so that in the course of fourteen days the culture might be inoculated upon sheep without danger, though where the culture had only been treated for twelve days, one-half the inoculated animals died. In these cultures, if maintained at a temperature between 42° and 43° C., the bacilli or bacteria survived for forty-six weeks, and furnished successful inoculations for protection against anthrax; after this period had passed, they died. If, however, it were found that the sheep had been inoculated with the culture fluid twenty-four days old, when again inoculated within twelve days with bacteria taken immediately from an animal sick with anrhrax, they died. If this second inoculation were made with the twelve-days-old culture instead of the blood, the sheep were found necessarily protected. He found that the inoculations should not be repeated too rapidly; at least twelve days should elapse between them, lest the effect be cumulative. Pasteur and his

assistants, Chamberland and Roux, usually inoculated animals upon the inner side of the thigh, or upon the ear, or injected the culture underneath the skin.

Cattle are not so easily affected as sheep. Pasteur anounced that with sheep 80 per cent. had an immunity lasting over one year. It has been found, however, that only certain animals are susceptible to the virus of anthrax. At this point it will be well to note the results of Pasteur's method in England.

Thus, in 1884, Lauder Brunton says, though we agree with Koch and Klein that immunity can be conferred by inoculation, this is an interestiong rather than a practical subject. He says, Pasteur has claimed that his method gives absolute immunity, and that it is harmless, but there is no doubt that when Pasteur performs his inoculations without any deaths, he works with cultures too weak to give any immunity. His inoculation fluid *vaccines* have been found variable. Thus, sometimes his *vaccin premier* has killed a flock of sheep, while the *vaccin deuxieme* has been inert. The objection to this method is that immunity can only be conferred by a percentage of loss from deaths greater than would result if the flock were turned upon a notoriously infected pasture. And further, this inoculation favors the spread of the disease by the formation of spores when any of the bacilli fall on the wool of the animal. The immunity thus given lasts, at the most favorable computation, no longer than one season. That it may be possible in the future to dis-

cover a method of obtaining immunity without too great a loss during the process must be allowed, but to consider it proven that we at present possess such a means in the method of inoculation described by Pasteur, can only lead to disappointment, etc.

Chauveau's Method.—Prof. Chauveau was one of the first to demonstrate that the presence of oxygen, which Pasteur alleged attenuated the virus, was really inert in this respect. He further showed that bacteria of anthrax would survive a temperature of 47° C., after exposure to it for two to four hours at least; they were rendered less harmful, but they were still virulent. His improved method was as follows:

He first took a drop of fresh infected blood from an animal suffering from anthrax, and then placed it in the culture flask which contained 20 grains of sterilized broth; there he retained it for two hours at 43° C.; it was then heated for three hours to 47° or 49° C. He further showed that flasks containing one or two litres could furnish sufficient culture virus to inoculate at least 8,000 sheep. He took one of these flasks with three openings, and filled it five-sixths full of sterilized broth. The middle opening was armed with a long tube which descended to the bottom of the flask; this tube, the outer edge of which was filled with cotton, was used for the entrance of air. Of the two side openings, one is used as an adductor tube, and the other is a drawn out cylinder for emptying the small flasks. By this last tube we introduce the

bacteria, in the proportion of one drop to ten grammes of culture fluid. After the introduction of the germ fluid the tube is closed and passed through the flame of the Bunsen burner. This last culture fluid is then placed in a thermostat at 35° to 37° C. The development is incomplete if the fluid be at rest, but when air is passed through it by means of the tube, abundant development takes place. In one week the germination is complete, and there is a rich development of spores, whose virulence the heat has attenuated. The best culture fluid is chicken broth, one part meat to four of water. The current of air should be very regular. The flask should be shaken night and morning. The nearer the temperature to 40° C., the better the culture. Third, from this large flask the little tubes used by Pasteur are filled. Some are placed in a water-bath, others in an air-bath and heated to 85° or 90° C. In order to get the first inoculation (*premier vaccin*), heat them to the highest point possible short of preventing proliferation. For the second inoculation (*deuxieme vaccin*), heat to a point two degrees less; usually 84° C. answers for the first, and 82° for the second.

Chamberland and Roux's Method.—Objections having been made to Chauveau's method that his attenuated virus does not retain its attenuation, Chamberland and Roux undertook to improve upon Toussaint's method. They took the beef-broth culture of the bacillus anthracis, after it had been neutralized

with potash, etc., and antiseptics, they placed the culture in an oven at 35° C. The growth of bacteria was greatest where the least antiseptic had been used, and diminished as the percentage of the antiseptic increased. The attenuated bacteria retained their attenuation. According to Chamberland and Roux, the test for the attenuation of the virulence according to any method, should be the absence of spurules in the filaments.

Koch, Gaffky, and Loeffler's Method.—These experimenters used the thermostat of D'Arsonval, which admits of a variation of temperature of only one-tenth to one degree. They also used the culture flask of Erlenmeyer. Each flask containing 20 c.c. of chicken-broth, neutralized by carbonate of soda. After the flasks had been inoculated they were placed in the thermostat at 42° to 43° C., and the degree of attenuation was found by experiments on the lower animals, such as mice, rabbits, and the like. They came to the conclusion that methods for the protective inoculation which had hitherto been recommended, were of doubtful advantage.

How to Prepare the Bacilli of Anthrax.—The bacilli may be stained with ordinary aniline dyes, and in various ways. It is best, however, to decolorize the tissues if they are in solid organs, and Gram's method is to be recommended for this purpose. Weigert's double staining method is also recommended.

I will now say a few words about the morphology of the microzymes found in infected

CHOLERA.

The comma bacillus, which Koch claims to be pathognomonic of cholera, is a slender body with a rounded extremity blended with the former. Much dispute has arisen in reference to this bacillus, Lewis, of India, claiming that a similar bacillus can be found in the secretion of the human mouth, while Finckler and Pryor claim that a similar comma bacillus may be found in the dejections of cholera nostras patients. But Koch has claimed, in reply to these last objections, that the two comma bacilli have a different growth in gelatine or potato, and that there are other morphological differences.

Koch's Method for making a diagnosis in epidemic cholera is as follows:* The intestines of Asiatic cholera patients show an infiltration with bacteria which are partly in the glands, and partly between the epithelium and the basement membrane. The intestinal contents, however, exhibit great variety in the form of bacteria. The bacillus of cholera is claimed to be smaller than that of tuberculosis, being about two thirds the length, but it is firmer and thicker than the latter, and has a slightly bent appearance. Sometimes the growth is doubled so that the bacillus is shaped like the letter S. This peculiar conformation

* Koch's Report at the Cholera Convention, July 26, '84.

is due to the union of two individuals. In pure cultures another form is recognized. This consists of rather long filaments similar to the spirilla of recurrent fever; so very similar are they that Koch thinks he would be unable to distinguish the two. Cultivated in meat broths the cholera bacilli increase with great rapidity, and are unusually active. They also grow abundantly and rapidly in milk, but do not cause it to curdle; it appears unchanged, but if a small drop be taken from the surface and examined microscopically, it will be found to teem with cholera bacilli. The cholera bacilli also grow well in blood-serum, and also in peptone-gelatin. The comma bacillus may be cultivated upon agar-agar. Cultures may also be made upon boiled potato when they develop growths like those of the bacilli of glanders. It seems that the vegetation of the bacilli in gelatine not only dissolves the latter, but thins the fluid and in so doing they form cup-like cavities on the surface; these peculiarities are supposed to distinguish comma bacilli from other bacilli. The comma bacilli germinate best at a temperature between 30° and 40° C.; below 17° the development is slow, and ceases at 16°. Koch believes that if the cholera bacilli are introduced from the intestine into a putrid fluid containing the productions of the decomposition of other bacteria, they will not develop well, but will soon die.

Nicati's and Rietsch's Method.—They took a Pasteur filter and pass an eight-day pure culture in

broth or gelatin through it. The filtrate was injected into the veins of a dog, which afterwards exhibited all the symptoms of cholera.

It is interesting to note here that Rochefontaine has swallowed the alvine dejections of a cholera patient made into five large, soft pills. These pills he took successfully with water. The pulse rose to 100, the skin became hot, he had slight nausea, dysuria, slight convulsion of the muscles of the legs, etc., and constipation for twenty-four hours. He then took a glass of alkaline water and felt well again.*

GLANDERS.

The bacterium associated with this disease has been called the bacillus malandriæ.

Loeffler's and Schutz's Method.—A solid culture is made from the blood-serum of a horse. This is then inoculated with the matter from a fresh tubercle. After an interval of three days the surface exhibits a number of small transparent spaces; in these there are small bacilli similar to those that occur in the lung, liver, spleen, etc. In staining these bodies, methyl-blue is used in a concentrated solution.

HOG CHOLERA.

Synonym: Swine-plague.

Opinions differ as to the form of the bacterium

* Comptes Rend. Tom. 99, Nov. 17, 1884, p. 845.

associated with this disease. Klein* holds that it is a bacillus of very small size; others that it is a micrococcus of the link form, called diplo-coccus. The last named form has been described by Pasteur.†

HYDROPHOBIA.

Pasteur has described in this disease a bacillus lyssæ. As in the former case, it is still a matter of dispute whether the micro-organism associated with the disease is a bacillus or a micrococcus. Pasteur adheres to the former view; others maintain the latter one.

Pasteur's Method of Inoculation.—The animal is trephined and the virus is injected, with an admixture of water, into the membranes of the brain, by a hypodermic syringe. He states that in fifteen to twenty days the animals die of rabies, and the diseased brain is then capable of producing hydrophobia in other animals. His statements are as follows:

First. If the virus of rabies is transferred from a dog to a monkey, and then to other monkeys, it gradually becomes weaker; and, if it be injected in this state into a dog, rabbit, or guinea pig, it remains in the same condition.

Second. On the other hand, the poison is in-

* The Report of Infectious Pneumo-enteritis of the Medical Office of the Privy Council, 1877–78, London.

† Pasteur and Thuillier, Vacc. de Rouges de Porc. Comp. Rend., Tom. 97, p. 1163.

creased if it be transferred from one rabbit to another, or from one guinea-pig to another. Now, in this intensified condition, the virus is inoculated upon a dog, in which case it invariably produces death. But, although the virulence of the poison may be thus increased by a transference from one rabbit to another, it is necessary to repeat the process several times if it has been attenuated by inoculation upon a monkey; and hence, Pasteur claims that he can render the animal insusceptible to the real disease. If, for example, it be desirable to make a dog refractory to the real disease, the virus is first inoculated upon several rabbits, but inoculated upon the dog at every successive inoculation upon a rabbit.*

Gibier's Method of Attenuation.—He exposes the virus of rabies to a very low temperature in order to weaken it. His method is as follows:

He takes a small drill and perforates the skull in the median line; the virus is then injected by means of a hypodermic syringe. The value of this method has not been satisfactorily determined.

Babes' Method for Staining.—He simply makes cover-glass preparations of the saliva, using the ordinary methyl-violet fluid; aniline-red fluids are also used. It is improbab'e that the bacteria in these cases have been successfully isolated.

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* Pasteur, Comp. Rend. Tom. 92, p. 1260.

Koch* contends that the organisms which Pasteur† has described were simply those that belong to septicæmia.

LEPROSY.

The bacterium of this disease has been called the bacillus lepræ by Hansen.‡ The bacilli are said to be organisms which do not give up in acid solutions the staining or color which they have previously taken. Bismarck-brown will not stain them, but the blue, violet, and red aniline colors will.

Baumgarten's Method.—He takes a saturated alcoholic solution of aniline-red and adds five drops of it to a watch-glass of distilled water. The cover-glasses are floated upon this liquid for six or seven minutes, with the bacilli upon them. They are then placed for fifteen seconds in acidulated alcohol. To decolorize the preparations, use the following preparation: Nitric acid, one part; alcohol, two parts. They are then again stained with a watery solution of methyl-blue, subsequently washed in water, saturated with absolute alcohol, clarified in oil of bergamot, and mounted in Canada balsam. The objects are then studied with an oil immersion lens. It is said that tubercular bacilli do not stain by this method. When staining the bacilli in sections, the latter should remain

* Ueber die Milz brand Impfung, Berlin, 1882, p. 5.

† Recherch. Sous la Rage, Comp. Rend., Tom. 98, 1884. Et Pasteur Comp. Rend., Tom. 98, No. 8, p 477, 1884.

‡ Virchow. Archiv., Band 79, 1880.

somewhat longer—twelve to fifteen minutes—in the staining fluid. They should be decolorized for thirty seconds in the solution, and washed three or four minutes in water, etc. The restaining with methyl-blue should occupy two or three minutes. The lepra bacilli, by this method, assume after a few minutes a red color. The bacilli of tuberculosis are colorless if present.

*Neisser's Method.**—Neisser stains in gentian-violet or methyl-violet. His culture method consists in taking a lepra tubercle, making cultures from it under proper precautions in blood-serum, sterilized, etc. The preparations are kept in an oven at from 35° to 39° C.

BACULLIS OF MALARIA (KLEBS).

Klebs' and Tomassi-Crudelli's Method.—The supposed infective material was taken from the air of a malarial district. The germs were collected by forcing the air against a glass plate standing at right angles to the air current. The plate was covered with glycerine-gelatin. From this plate fractional cultures were made in fluid media of various kinds. After passing these pure cultures through plaster-of-Paris filters, animals were inoculated, first with the unfiltered material, and then with the filtrate. The latter exhibited only a slight rise of temperature; the former had typical malarial fever. In order to obtain the

* Neisser, Virchow, Archiv., Band 84, 1884.

germs from the earth, they took a porcelain vessel of large size and placed it upon a sand bath. The vessel was then well lined with damp earth; it was kept moistened from time to time with a little water. Upon this prepared earth was placed a metal box containing the earth to be examined; the bottom of it was perforated with numerous openings. The whole apparatus was then kept at a temperature of from 30° to 35° C. Fractional cultures were then made from the earth in the box, and animals were inoculated. It was found that the germs would not pass through the plaster-of-Paris filters.*

Richard's Method.—He makes a direct examination of the blood taken from a patient's finger. This method is the best to be employed for studying the microbe in its movements. In order to see the bacteria in cases where there are very few present, he destroys the normal red corpuscle by adding to a drop of the blood a drop of acetic acid; the bacteria are then readily brought into view. He finds that the microbe has a special affinity for the red corpuscle in which it is developed.†

SYPHILIS.

In this disease also, as in others that have been mentioned, there is a dispute as to whether the special bacterium is a micrococcus or a bacillus.

* Tomassi-Crudelli, Archiv für Exper. Path. und Phar., Band 12, Heft 3, p. 225.

† Comp. Rend. No. 8, 1882.

Birsch-Hirschfeld's Method.—He takes syphilitic deposits or lesions that have been hardened in absolute alcohol, then stains them in a concentrated solution of aniline-red, washes in distilled water, and mounts in the usual way.†

TUBERCULOSIS.

The bacillus tuberculosis is the special parasite which has been connected with the disease by Koch. Tubercular bacilli resemble the bacilli of leprosy in so far as they will retain their color even after immersion in strong acids, and this quality is said to be enjoyed in common with them by the bacilli of leprosy only, as we have seen. These bacilli also often exhibit round or oval spores in their interior. The best method, which has been generally accepted for staining bacilli, is that of Ehrlich.

Ehrlich's Method.—He takes a small amount of sputum, presses it between two cover-glasses, and separating them, passes them, with the surface turned upward, through the gas flame. He then turns them with the preparation on the lower side and floats them in a watch-glass filled with gentian-violet, methyl-violet, or his fuchsine aniline oil solution, allowing them to remain there for from fifteen minutes to half an hour. He then heats the whole over a flame until it steams for one minute, then washes in a

† Birsch-Hirschfeld, Centralbl. für die Med. Wissen., 1882 No. 33.

solution of nitric acid, 33 per cent., so that the color fades out of the matrix, the bacilli alone retaining it, then washes in distilled water, dries, passes through absolute alcohol, and mounts in balsam. In order to make the picture clearer he then restains the decolorized matrix. This is best done by some contrasting color, such as Bismarck-brown, vesuvine, or the like.

To Stain Sections.—Sections should remain in the staining fluid for twenty-four hours, and for two or three minutes in the acid mixture. Wash well in water several times, and then mount in the ordinary way.

Ziehl's Method.—In this method carbolic acid is substituted for aniline oil and caustic potash. This method consists in preparing, as in Ehrlich's method, but omitting the decolorization, by the nitric acid solution. In place of aniline oil he uses pyrogallic acid and resorcin, and he was the first to show that the tubercular bacillus does not need an alkaline staining fluid. Still later, Dr. Pryor showed that Ehrlich's method was not alkaline, but neutral, and that oil of turpentine might take the place of the aniline oil.

Among other methods are those of Fraenzel* and of Rindfleisch.

Baumgarten's Culture Method.—Baumgarten took a small fragment from a human nodule and introduced it with antiseptic precautions into the anterior chamber of the eye of the living rabbit. Here it increased in size. He then removed this particle and

* Berliner Klin. Wochensch., 1882, No. 145.

placed it into the anterior chamber of the eye of another rabbit, and in six or eight days in a third rabbit. This method requires no trouble and needs no special apparatus, and a pure culture is thus obtained outside of the living body.

Reinstadler's Culture Method.—He took a piece of tubercular lung, placed it in a sterilized vessel with some sand that had been heated red hot. To this material he added a quantity of Bergman's fluid, which had been thoroughly super-heated. He filtered this liquid and sealed for use. Some test-tubes were then cleansed by boiling in nitric and sulphuric acid, and then in alcohol; they were then super-heated in a spirit flame and plugged with carbolized cotton; then they were filled with a sterilized pipette with 30 c. c. of Bergman's fluid, and the whole is sterilized by heat, and cooled.

Celli and Guarnieri's Method.—To examine the air used by consumptive patients, they took a large tin cylinder, bent at the top, and terminating below in a conical extremity whose apex contained a small pointed tube, the free end of which terminated in a wide cone of tin, into which was introduced a cone of copper bent at its apex, and the surface of which was coated with Koch's gelatine. The gas flame caused a current of air to pass over the gelatin surface; a gas jet was placed in the lower part of the large tin cylinder. The gelatine was kept at an even temperature of 30° to 40° C. Examinations were made in the

usual way. They also made further experiments by requiring patients to breathe repeatedly for twenty-four hours upon a wooden dish coated with Koch's gelatine, and covered by a watch-glass. They also used a variety of other methods and experimented with sputum. They found that the sputum, so long as it is moist, does not evolve any specific bacilli.

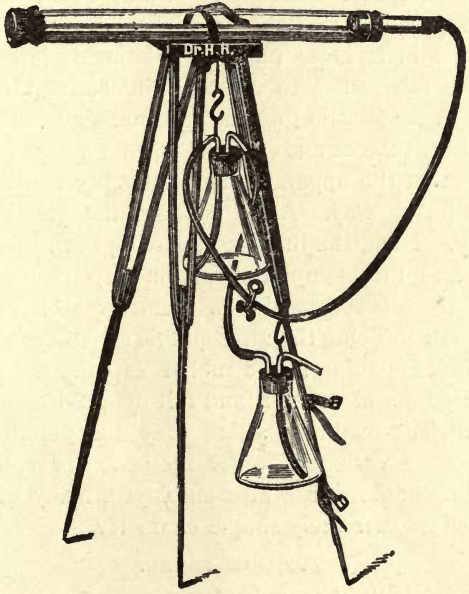

Fig. 19.

Hesse's apparatus for examining ordinary atmospheric or contaminated air consists of a hollow glass

cylinder, 18 inches in length and 2½ in diameter. One end of the tube is fitted with a rubber cap perforated by a minute orifice. The other end is occupied by a perforated India-rubber cork, pierced by a short piece of glass tubing. To the tubing is attached a litre flask, that is again similarly connected with another litre flask. The cylinder is sterilized by superheating in the steam apparatus, or by washing with a $\frac{1}{1000}$ solution of the bichloride of mercury. Then a thin layer of nutrient gelatine is introduced into the tube, so as to make a thin coating along the floor. After shutting off the external air by clamping the rubber tube at one end and putting a cap of cotton in the other the apparatus is thoroughly sterilized in the ordinary way. After cooling, the gelatine will solidify. Next, the litre flasks having been filled with water and the clamp and cotton cap removed, the flasks are allowed to siphon themselves off drop by drop. In so doing the atmospheric air enters through the minute opening in the rubber cap and the atmospheric germs also enter and fall by gravitation upon the nutrient medium, where they are permitted to develop. A variety of other methods for collecting atmospheric germs are also employed, many of them being of an extremely simple character.

TYPHOID FEVER.

The bacilli of typhoid fever were first named by Brautlecht.* If Gram's method is used the bacilli are

* Virchow, Archiv., 1880, p. 80.

decolorized. In this case, as in the foregoing, there is a difference of opinion as to whether the bacterium is a micrococcos or a bacillus.

Letzerich's Method.—His plan was to take fresh tissues, or those that were partly hardened, make sections and clarify them with weak solutions of caustic potash or carbonate of soda, or even in diluted acetic acid. He mounted them in glycerine and water.

Rindfleisch's Method.—He took water from a suspected well, placed a drop upon a slide, colored it with a solution of methyl-violet, washed in water, dried, and mounted in Canada balsam. Some deep blue rod bacteria were brought into view. He then inoculated with the water gelatin made of human flesh, under proper precautions. The gelatin was rapidly dissolved by the culture.*

CHICKEN CHOLERA.

Klein believes that the organism Pasteur has described is a bacterium termo of putrefaction. Others call it a micrococcus or diplo-coccus. For a culture fluid in this case Pasteur employed a meat or chicken broth, neutralized with carbonate of potash, and sterilized at a temperature of 100° to 115° C. After cultivating these microbes for a few days, he inoculated a chicken, producing the disease in a mild form.

* Sitz. Bericht der Phys. Med. Gesell. zu Wurz, 1882, p. 133.

DIPHTHERIA.

The organism in this case has been called the micrococcus diphtheriticus by Cohn.

The same difference of opinion occurs among investigators as to whether the organism in this case is a spherical- or a rod-shaped body. Klein and others believe that the spherical bodies are the germs of the disease.

Loeffler's Method.—He takes 30 c. c. of concentrated alcoholic methyl-blue solution, 100 c. c. of caustic potash solution, and in the proportion of one to 1000 of water. The sections remain only for a few moments in this solution, which gives a deep stain to all known bacteria. The sections are then placed for a short time in one-half-per-cent. acetic acid solution in the ordinary way, and mounted according to ordinary rules. This method demonstrated to Loeffler two forms; one, the chain bacterium, which he was able to cultivate in blood-serum and on cooked potato; and the other, a bacillus that grew at 37° C., in a mixture of three parts of calves' or sheep's serum to one of neutralized beef broth, one per cent. peptone, one per cent. beet sugar, and 1 per cent. salt. These bacilli were regarded by Loeffler as active promoters of the disease, while the spherical forms he thought were unimportant.

ERYSIPELAS.

Fehleisen's Method.—The material he takes from

an erysipelatous blister, together with a part of the skin, washes it with ether, and sprays it with a sublimate solution. The diseased tissue is then put in nutrient gelatine or blood-serum, and is cultivated in the ordinary way. Having obtained a culture, he inoculated a woman 58 years old, who was dying with multiple sarcomata of the skin. A typical case of erysipelas resulted. He found, as he thought, that these inoculations in other cases favorably influenced the progress of neoplasms. This was true in a case of cancer, another of lupus, and another of sarcoma. A three-per-cent. solution of carbolic acid destroys the poison.*

GONORRHŒA.

Neisser's Method.— To stain the micrococcus gonorrhœæ, he spreads the secretion thinly upon a sterilized coverglass. This is best done by placing a drop between two glasses, after which they are drawn apart. The preparation is dried in the air, heated up to 120° C., and allowed to remain at that temperature for one or two hours. The specimen should be well stained. There is no staining fluid so good as methyl-blue, which stains the bodies rapidly. The coverglasses remain in the dye from twelve to twenty-four hours. They are best studied with a high immersion power.

* Fehleisen, Sitz. Bericht, Med. Phys. Gesell., Wurzburg, 1883, No. 1.

To produce a culture, prepare neutralized beef extract gelatine, or neutralized blood-serum, etc. As for inoculation, it has been shown that dogs have immunity, also rabbits; but when the pure culture was injected into the urethra of a man 46 years old, he got up a true gonorrhœa.*

CROUPOUS PNEUMONIA.

Friedlaender's Method.†—In order to make a culture, Friedlaender takes portions of lung from a patient who has died from the disease, removing them with a sterilized wire, and cultivates in blood-serum according to Koch's plan. The pure cultures have a characteristic nail shape in gelatine. The micrococci are characterized in may cases by a peculiar capsule. It is thought by some that this is due to the mode of preparation and staining. In staining tissues the sections should remain for one hour in the aniline gentian-violet solution at 45° C. They should then be washed in the iodo-iodide of potash solution, consisting of iodine, 1 part; iodide of potassium, 2 parts; and water, 300 parts; passed through absolute alcohol and oil of cloves, and mounted in Canada balsam.

RECURRENT FEVER.

The spirilla are different from all other bacteria, in so far as they are destroyed by acids, alkalies, and

* Neisser, Deutsche Med. Wochenblatt, 1884, p. 279.

† Med. Bericht, Clin. Wochenschrift, 1883, No. 48, p. 752.

even by distilled water. No one has succeeded in staining them but Koch, who used brown aniline, and succeeded in photographing them.

*Friedlaender's Method.**—His method of obtaining these bodies is to take blood from a patient by means of a cupping-glass and allow it to coagulate; the spirilla remain on the surface of the clot and retain their characteristics for days. They occur in the blood only during the febrile attacks.

YELLOW FEVER.

Friere's Method.—This was to cultivate the organisms obtained from the blood by general methods, and to attenuate the cultures by heat in the manner employed by Pasteur for anthrax. In 1883 he received permission from the emperor of Brazil to inoculate some of his subjects. He operated several hundred times, and is said to have produced the disease in a mild form. The immunity conferred seemed to be perfect*.

VARIOLA.

The micrococcus variolæ et vaccinæ, according to the name given by Cohn†, is the cause of the disease,

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* Koch, Deut. Med. Wissen., No. 19, 1879; Obermeier, Berlin. Klin. Wochenbl., 1873, pp. 152 und 391.

* Friere et Rabougeon, Comp. Rend. Acad. de Science de Paris, Tom. 99, Nov. 10, 1884.

† Virchow Archiv., 1872.

but as yet no successful cultures or inoculations have been performed. But, in view of successful experiments, an English Company, the Worshipful Company of Grocers, offered a prize of five thousand dollars to the discoverer of a method by which the vaccine contagion may be cultivated apart from the animal body. The prize was open to universal competition, British and foreign, and the paper was to be submitted before December 31, 1886. The committee who had the matter in charge were John Simon, Burdon Sanderson, George Buchanan, and others. Their report has not yet been read.

In concluding this brief summary, it will be observed that of the sixteen diseases enumerated, in at least eight the form of the microphyte is in dispute. It is safe to say, however, that there is even less certainty in the matter than this ratio indicates.

Micrococcus pneumonicus.
" diphtheriticus.
" gonorrhœicus.
" of urine.
" prodigiosus.
" of vaccine virus.
Spirochaete buccalis (mouth).
" obermeieri (from typhus recurrens).
Saccharomyces cerevisii (upper yeast).
" " (lower yeast).
(These two are unstained; price, 75 cts. each.)
Satcina ventriculi, from stomach.
Oidium albicans, from mouth.
Achorion schoenleini (favus).

T. H. McAllister, manufacturing optician, of 49 Nassau St., New York City, has a still larger and almost complete series of the stained and unstained bacteria found in the several infective diseases.